

Planta Medica

Journal of Medicinal Plant and Natural Product Research

Editor-in-Chief

Luc Pieters, Antwerp, Belgium

Senior Editor

Adolf Nahrstedt, Münster, Germany

Review Editor

Matthias Hamburger, Basel, Switzerland

Editors

Wolfgang Barz, Münster, Germany
 Rudolf Bauer, Graz, Austria
 Veronika Butterweck, Gainesville FL, USA
 João Batista Calixto, Florianopolis, Brazil
 Thomas Efferth, Mainz, Germany
 Jerzy W. Jaroszewski, Copenhagen, Denmark
 Ikhlas Khan, Oxford MS, USA
 Wolfgang Kreis, Erlangen, Germany
 Irmgard Merfort, Freiburg, Germany
 Kurt Schmidt, Graz, Austria
 Thomas Simmet, Ulm, Germany
 Hermann Stuppner, Innsbruck, Austria
 Yang-Chang Wu, Taichung, Taiwan
 Yang Ye, Shanghai, China

Editorial Offices

Claudia Schärer, Basel, Switzerland
 Tess De Bruyne, Antwerp, Belgium

Advisory Board

Giovanni Appendino, Novara, Italy
 John T. Arnason, Ottawa, Canada
 Yoshinori Asakawa, Tokushima, Japan
 Lars Bohlin, Uppsala, Sweden
 Gerhard Bringmann, Würzburg, Germany
 Reto Brun, Basel, Switzerland
 Mark S. Butler, S. Lucia, Australia
 Ihsan Calis, Ankara, Turkey
 Salvador Cañigueral, Barcelona, Spain
 Hartmut Derendorf, Gainesville, USA
 Verena Dirsch, Vienna, Austria
 Jürgen Drewe, Basel, Switzerland
 Roberto Maffei Facino, Milan, Italy
 Alfonso Garcia-Piñeres, Frederick MD, USA
 Rolf Gebhardt, Leipzig, Germany
 Clarissa Gerhäuser, Heidelberg, Germany
 Jürg Gertsch, Zürich, Switzerland
 Simon Gibbons, London, UK
 De-An Guo, Shanghai, China
 Leslie Gunatilaka, Tucson, USA
 Solomon Habtemariam, London, UK
 Andreas Hensel, Münster, Germany
 Werner Herz, Tallahassee, USA
 Kurt Hostettmann, Geneva, Switzerland
 Peter J. Houghton, London, UK
 Jinwoong Kim, Seoul, Korea
 Gabriele M. König, Bonn, Germany
 Ulrich Matern, Marburg, Germany
 Matthias Melzig, Berlin, Germany
 Dulcie Mulholland, Guildford, UK
 Eduardo Munoz, Cordoba, Spain
 Kirs-Maria Oksman-Caldentey, Espoo, Finland
 Ana Maria de Oliveira, São Paulo, Brazil
 Nigel B. Perry, Dunedin, New Zealand
 Joseph Pfeilschifter, Frankfurt, Germany
 Peter Proksch, Düsseldorf, Germany
 Thomas Schmidt, Münster, Germany
 Volker Schulz, Berlin, Germany
 Hans-Uwe Simon, Bern, Switzerland
 Leandros Skaltsounis, Athens, Greece
 Han-Dong Sun, Kunming, China
 Benny K. H. Tan, Singapore, R. of Singapore
 Ren Xiang Tan, Nanjing, China
 Deniz Tasdemir, London, UK
 Nunziatina de Tommasi, Salerno, Italy
 Arnold Vlietinck, Antwerp, Belgium
 Angelika M. Vollmar, München, Germany
 Heikki Vuorela, Helsinki, Finland
 Jean-Luc Wolfender, Geneva, Switzerland
 De-Quan Yu, Beijing, China

Publishers

Georg Thieme Verlag KG
Stuttgart · New York
 Rüdigerstraße 14
 D-70469 Stuttgart
 Postfach 30 11 20
 D-70451 Stuttgart

Thieme Publishers
 333 Seventh Avenue
 New York, NY 10001, USA
 www.thieme.com

Reprint

© Georg Thieme Verlag KG
 Stuttgart · New York
 Reprint with the permission
 of the publishers only

Report Documentation Page			Form Approved OMB No. 0704-0188		
Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.					
1. REPORT DATE SEP 2011		2. REPORT TYPE		3. DATES COVERED 00-00-2011 to 00-00-2011	
4. TITLE AND SUBTITLE Triterpenoidal Saponins: Bioactive Secondary Metabolites from Zygophyllum coccineum		5a. CONTRACT NUMBER			
		5b. GRANT NUMBER			
		5c. PROGRAM ELEMENT NUMBER			
6. AUTHOR(S)		5d. PROJECT NUMBER			
		5e. TASK NUMBER			
		5f. WORK UNIT NUMBER			
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Mississippi, USDA, Agricultural Research Service, Natural Products Utilization Research Unit, University, MS, 38677		8. PERFORMING ORGANIZATION REPORT NUMBER			
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)		10. SPONSOR/MONITOR'S ACRONYM(S)			
		11. SPONSOR/MONITOR'S REPORT NUMBER(S)			
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Phytochemical investigation of the aerial parts of Zygophyllum coccineum L. led to the isolation of nine ursane-type triterpene saponins (1?9), including the new one; zygophylloside S (1), together with a known flavonoid glycoside (10) and a sterol glycoside (11). The isolated compounds were tested for antifungal activity against several important plant pathogens and for insecticidal activity against two important mosquito species. Among the isolated compounds 1, 3, 5, 6, and 9 showed 32?77% fungal growth inhibition at a concentration of 30 &#956;M against Phomopsis viticola. Compound 9 showed 90% and 80% mosquitocidal activity at 3.1 &#956;g/0.5&#956;L against Aedes aegypti and Culex quinquefasciatus, respectively.					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Same as Report (SAR)	18. NUMBER OF PAGES 4	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

Triterpenoidal Saponins: Bioactive Secondary Metabolites from *Zygophyllum coccineum*

Elham Amin^{1,5}, Seham S. El-Hawary², Magda M. Fathy², Rabab Mohammed¹, Zulfiqar Ali⁵, Nurhayat Tabanca³, David E. Wedge³, James J. Becnel⁴, Ikhlas A. Khan⁵

¹ Faculty of Pharmacy, Beni Suef University, Beni Suef, Egypt

² Faculty of Pharmacy, Cairo University, Cairo, Egypt

³ U. S. Department of Agriculture, Agricultural Research Service, Natural Products Utilization Research Unit, University of Mississippi, University, MS, USA

⁴ USDA, ARS, Center for Medical, Agricultural, and Veterinary Entomology Gainesville, Gainesville, FL, USA

⁵ National Center for Natural Products Research, University of Mississippi, University, MS, USA

Abstract

Phytochemical investigation of the aerial parts of *Zygophyllum coccineum* L. led to the isolation of nine ursane-type triterpene saponins (**1–9**), including the new one; zygophylloside S (**1**), together with a known flavonoid glycoside (**10**) and a sterol glycoside (**11**). The isolated compounds were tested for antifungal activity against several important plant pathogens and for insecticidal activity against two important mosquito species. Among the isolated compounds **1**, **3**, **5**, **6**, and **9** showed 32–77% fungal growth inhibition at a concentration of 30 μ M against *Phomopsis viticola*. Compound **9** showed 90% and 80% mosquitocidal activity at 3.1 μ g/0.5 μ L against *Aedes aegypti* and *Culex quinquefasciatus*, respectively.

Key words

Zygophyllum coccineum L. · Zygophyllaceae · ursane-type triterpene glycosides · zygophylloside S · antifungal activity · insecticidal activity

Supporting information available online at <http://www.thieme-connect.de/ejournals/toc/plantamedica>

Saponins are widely distributed in the plant kingdom and have a wide range of biological properties. Several investigations have reported the anti-inflammatory, antibacterial, antifungal, antiparasitic, antiviral, hemolytic, and cytotoxic activities of saponins [1]. As a part of a research program aimed at identifying new natural fungicides and insecticides, *Z. coccineum* L., a saponin-rich plant, was chosen for further investigation.

Z. coccineum L., Zygophyllaceae, grows wild in Egyptian deserts [2]. It has been used in traditional medicine as antihelminthic, diuretic, antidiabetic, antiasthma, antigout, antirheumatic, and antihypertension agents [3–5].

Zygophylloside S (**1**) was isolated as a white powder from the *n*-butanol-soluble part of the methanol extract by repeated flash and gravity column chromatography over normal and reversed phase (RP-18) silica gel. The HR-ESI-MS exhibited an $[M + Na]^+$ ion at $m/z = 803.4166$ (calcd. 803.4097) that is consistent with the molecular formula $C_{41}H_{64}O_{14}$. The 1H -NMR spectrum of **1** displayed resonances for four tertiary methyls [$\delta_H = 0.88$ (s), 1.06 (s),

1.10 (s), 1.14 (s)], two secondary methyls [$\delta_H = 0.82$ (d, $J = 6.4$ Hz) and 1.23 (d, $J = 6.0$ Hz)], an olefin proton [$\delta_H = 6.01$ (s)], and an oxygenated methine [$\delta_H = 3.15$ (dd, $J = 12.0, 4.0$ Hz)]. The ^{13}C -NMR spectrum showed 41 resonances, including characteristic ones at $\delta_C = 180.4$ and 178.3 due to two carboxy groups and at $\delta_C = 134.4$ and 129.3 due to a double bond. The DEPT-135 experiment allowed differentiation of the 41 carbon resonances into six methyl, 11 methylene, 16 methine, and 8 quaternary carbons, of which 6 methyl, 9 methylene, 7 methine, and 8 quaternary carbons were attributed to the aglycon moiety. This data indicated an ursane-type triterpene skeleton for the aglycon, with a classical olefin bond at C-12, and two carboxy groups at C-27 and C-28, corresponding to quinovic acid [6]. In addition, the 1H - and ^{13}C -NMR spectra showed resonances for two sugar units, assignable to β -glucopyranose [$\delta_H = 4.76$ (d, $J = 7.6$ Hz, H-1'); $\delta_C = 105.3$ (C-1'), 84.2 (C-2'), 78.5 (C-3'), 71.9 (C-4'), 78.2 (C-5') and 63.1 (C-6')] and α -arabinopyranose [$\delta_H = 5.14$ (d, $J = 6.8$ Hz, H-1''); $\delta_C = 106.9$ (C-1''), 74.0 (C-2''), 74.6 (C-3''), 69.5 (C-4'') and 67.3 (C-5'')]. The long-range HMBCs observed from H-1' to C-3 and from H-1'' to C-2' revealed that the glucose was linked at C-3 and the arabinose at C-2'. The aglycone was recognized from the aforementioned 1H - and ^{13}C -NMR data (Table 1) as 3 β -hydroxyurs-12-ene-27,28-dioic acid. The assignment of the 1H - and ^{13}C -NMR data was facilitated by comparison with those of *Zygophyllum* saponins [6–11] and confirmed by HMQC, HMBC (Fig. 1), and COSY spectra. Accordingly, zygophylloside S (**1**) was elucidated as 3-*O*-[α -L-arabinopyranosyl-(1 \rightarrow 2)- β -D-glucopyranosyl] quinovic acid [12].

By comparing their spectral and physical data with those of known compounds reported in the literature [6–11, 13], compounds **2–11** were characterized as 3-*O*-[β -D-(2-*O*-sulphonyl)-quinovopyranosyl] quinovic acid (**2**), 3-*O*-[β -D-glucopyranosyl] quinovic acid (**3**), 3-*O*-[β -D-glucopyranosyl] quinovic acid-28-*O*- β -D-glucopyranosyl ester (**4**), 3-*O*-[β -D-quinovopyranosyl] quinovic acid-28-*O*- β -D-glucopyranosyl ester (**5**), 3-*O*-[α -L-arabinopyranosyl-(1 \rightarrow 2)- β -D-quinovopyranosyl] quinovic acid (**6**), 3-*O*-[α -L-arabinopyranosyl-(1 \rightarrow 2)- β -D-quinovopyranosyl] quinovic acid-28-*O*- β -D-glucopyranosyl ester (**7**), 3-*O*-[β -D-(2-*O*-sulphonyl)quinovopyranosyl] quinovic acid-28-*O*- β -D-glucopyranosyl ester (**8**), and 3-*O*-[β -D-(2-*O*-sulphonyl)glucopyranosyl] quinovic acid (**9**), isorhamnetin-3-*O*-rutinoside (**10**), and β -sitosterolglucoside (**11**).

The antifungal activity of the compounds was investigated against *Colletotrichum acutatum*, *C. fragariae*, *C. gloeosporioides*, *Botrytis cinerea*, *Phomopsis obscurans*, *P. viticola*, and *Fusarium oxysporum* and was observed against *P. viticola* after 144 h exposure. Among the isolated compounds, compound **6** showed 76.9% \pm 3.7% fungal growth inhibition at 30 μ M, followed by compounds **1** (55.6% \pm 4.0%), **5** (54.1% \pm 7.7%), **3** (44.3% \pm 6.2%), and **9** (31.5% \pm 7.0%) (Fig. 2), while captan used as the standard showed growth inhibition of 99.9% \pm 0.3%.

Isolated compounds were subjected to a high-throughput larval bioassay with *Aedes aegypti* and evaluated for adult toxicity against *A. aegypti* and *Culex quinquefasciatus*. Although no mortality was observed with any of the compounds against first instar larvae of *A. aegypti*, compound **9** exhibited significant adult mortality of 90% and 80% at 3.1 μ g/0.5 μ L concentration against *A. aegypti* and *C. quinquefasciatus*, respectively. This is the first report of the antifungal and insecticidal activity of compounds **1**, **3**, **5**, **6**, and **9**.

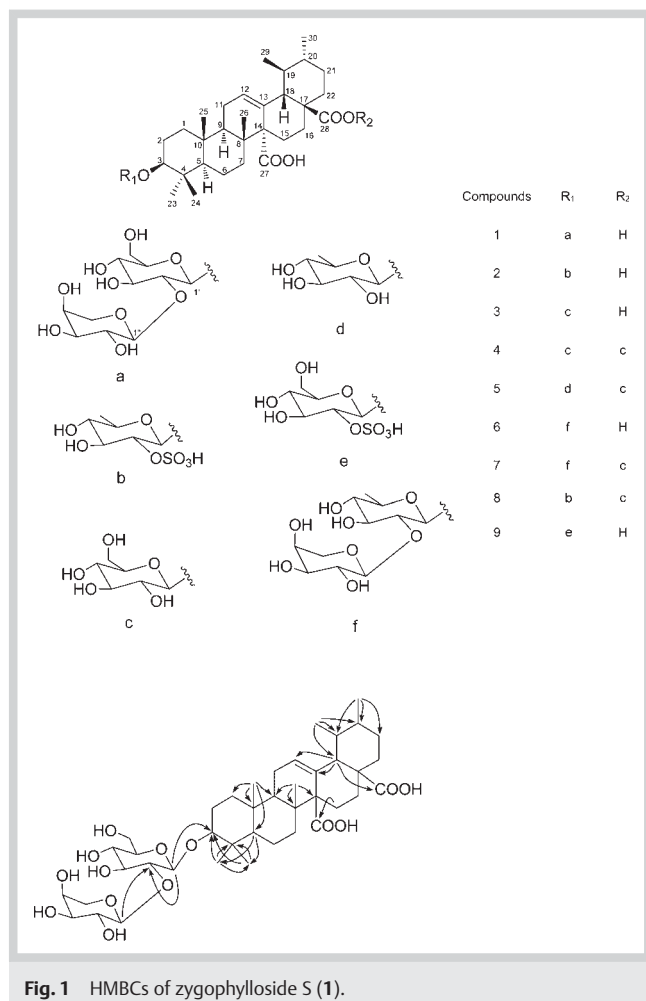


Fig. 1 HMBCs of zygophylloside S (1).

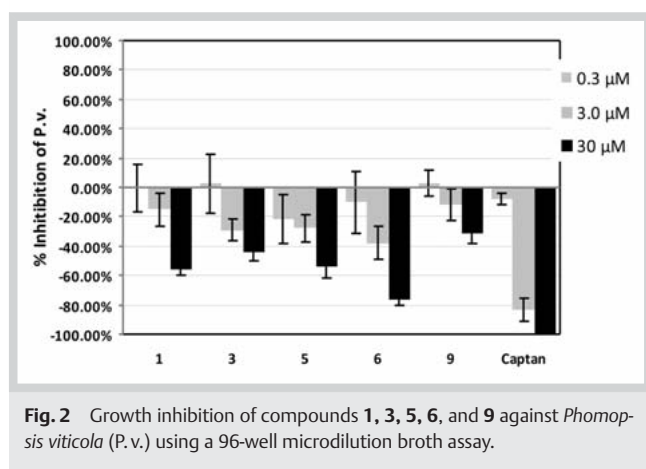


Fig. 2 Growth inhibition of compounds 1, 3, 5, 6, and 9 against *Phomopsis viticola* (P.v.) using a 96-well microdilution broth assay.

Materials and Methods

The aerial parts of *Z. coccineum* L. were collected from the eastern desert in Egypt in May 2008 and identified by Dr. M. Elgebaly, Prof. of Taxonomy, Faculty of Science, Cairo University. A voucher specimen has been deposited at the Herbarium of Faculty of Science, Beni Suf University.

Table 1 ¹H- and ¹³C-NMR data for zygophylloside S (1) in pyridine-*d*₅.

Position	δ _C	δ _H ^a	Position	δ _C	δ _H ^a
1	39.4	1.51, 1.02	22	37.8	1.40, 1.69
2	27.1	1.86, 1.95	23	28.0	1.14 s
3	89.0	3.15 dd (12.0, 4.0)	24	16.9	1.06 s
4	39.8	–	25	16.8	0.88 s
5	56.1	0.90	26	19.2	1.10 s
6	18.9	1.51, 1.31	27	178.3	–
7	37.4	1.98, 1.86	28	180.4	–
8	40.3	–	29	18.6	1.23 d (6.0)
9	47.4	2.67 dd (11.2, 5.0)	30	21.7	0.82 d (6.4)
10	37.3	–	1'	105.3	4.76 d (7.6)
11	23.6	1.90, 2.12	2'	84.2	4.06 t like (8.6)
12	129.3	6.01 s	3'	78.5	4.25 t like (8.6)
13	134.4	–	4'	71.9	4.20 t like (8.6)
14	57.1	–	5'	78.2	3.87 m
15	25.8	2.35, 2.61	6'	63.1	4.36, 4.52 dd (15.2, 3.6)
16	26.7	2.23, 2.24	1''	106.9	5.14 d (6.8)
17	49.0	–	2''	74.0	4.54 t like (8.0)
18	55.2	2.80 d (11.3)	3''	74.6	4.17
19	38.1	1.40	4''	69.5	4.30
20	39.7	0.80	5''	67.3	3.75 d (11.3), 4.36
21	30.9	1.30, 1.40			

Note: δ values are in ppm; J values (in Hz) are in parentheses. ^a Multiplicity is not clear for some signals due to overlapping

The dried, powdered aerial parts of *Z. coccineum* (2 kg) were extracted with 80% methanol (10 × 4 L) and evaporated under reduced pressure to give a residue (300 g). A part (150 g) was dissolved in water and sequentially fractionated with petroleum ether, chloroform, ethyl acetate, and *n*-butanol. The *n*-butanol extract (20 g) was chromatographed over a silica gel column (600 g, 8 × 60 cm) by using EtOAc/CHCl₃/MeOH/H₂O 15:8:4:1 (10 L) and 6:4:4:1 (6.5 L) to yield seven main fractions (A–G). Fraction D (300 mg) was chromatographed on a Biotage system [SP-1 (40 + M), C18 column, MeOH/H₂O 3:2 (2 L)] followed by silica gel CC [20 g, 1 × 35 cm, CHCl₃/MeOH/H₂O 4:1:0.1 (150 mL)] to afford 1 (9 mg).

Zygophylloside S (1): White powder; [α]_D²⁰: +0.050 (*c* 0.01, MeOH); IR (NaCl): ν_{max} = 3368 (OH), 1687, 1224 (CO) cm⁻¹; HR-EI-MS (positive-ion mode): *m/z* = 803.4166; ¹H- and ¹³C-NMR spectroscopic data: see Table 1.

Determination of sugars of 1

Compound 1 (3 mg) was heated with 2 N HCl at 95 °C for 3 h. The mixture was then neutralized with NH₄OH and extracted with EtOAc (2 × 2 mL). The aqueous layer residue was dissolved in pyridine and 0.1 M cysteine methyl ester hydrochloride in pyridine was added. The reaction mixture was heated at 60 °C for 1 h. An equal volume of phenyl isothiocyanate in pyridine was added and heated at 60 °C for 1 h. The mixture was filtered and analyzed by reversed-phase HPLC [Waters Alliance 2695, equipped with photodiode array detector, and Luna C18 column (150 × 4.6 mm, 5 μm particle size; Phenomenex, Inc.)], using acetonitrile + 0.1% acetic acid (A) and water + 0.1% acetic acid (B) in a gradient mode: A/B 10/90 for 20 min and A/B 55/45 for the next 25 min, at a rate of 1 mL/min. The response was detected at 250 nm. The standard sugar derivatives were prepared and analyzed identically. L-Arabinose and D-glucose were identified by comparison of the reten-

tion times of their derivatives with those of authentic sugar samples [L-arabinose: 13.4 min (minor)/15.5 min (major); D-arabinose: 13.3 min (minor)/15.7 min (major); D-glucose: 12.3 min (minor)/15.0 min (major); L-glucose: 12.8 min (minor)/14.9 min (major)].

Antifungal assay

Isolated compounds were evaluated using a 96-well microbioassay system for antifungal activity against *Colletotrichum acutatum*, *C. fragariae*, *C. gloeosporioides*, *Botrytis cinerea*, *Phomopsis obscurans*, *P. viticola*, and *Fusarium oxysporum* [14]. The technical-grade commercial fungicide captan (98%, Chem Service, Inc.) was used as a standard.

Mosquitocidal assay

Isolated compounds were subjected to a high-throughput larval bioassay with *Aedes aegypti* and evaluated for adult toxicity against *A. aegypti* and *Culex quinquefasciatus* [15, 16]. Permethrin (55% *cis* and 45% *trans*; Chem Service, Inc.) was used as a standard.

Supporting information

Detailed protocols for biological assays, general experimental procedures, thorough extraction/isolation procedures, and NMR spectra for compound **1** are available as Supporting Information.

Acknowledgements

▼ We thank the Egyptian government for a fellowship through the Ministry of Higher Education and Scientific Research. This study was also supported by a grant from the Deployed War-Fighter Protection Research Program (DWFP), the U.S. Department of Defense through the Armed Forces Pest Management Board (AFPMB), and the USDA ARS NPURU.

References

- 1 Sparg SG, Light ME, Staden JV. Biological activities and distribution of plant saponins. *J Ethnopharmacol* 2004; 94: 219–243
- 2 El-Hadidi MN. Flora des Iranischen Hochgebirges und der umrahmen-den Gebirge, Vol. 98. Graz: Akademische Druck- und Verlagsanstalt; 1972: 28
- 3 Saber AH, El-Moghazy AM. *Zygophyllum coccineum*. V. The chemistry of the leaf and stem. *J Pharm Sci* 1960; 1: 135
- 4 Eskander EF, Won JH. Hypoglycaemic and hyperinsulinemic effects of some Egyptian herbs used for treatment of diabetes mellitus (Type II) in rats. *Egypt J Pharm Sci* 1995; 36: 331–341

- 5 Gibbons S, Oriowo MA. Antihypertensive effect of an aqueous extract of *Zygophyllum coccineum* L. in rats. *Phytother Res* 2001; 15: 452–455
- 6 Poellmann K, Gagel S, Elgamal MHA, Shaker KH, Seifert K. Triterpenoid saponins from the roots of *Zygophyllum* species. *Phytochemistry* 1997; 44: 485–489
- 7 Ahmed VU, Uddin GS, Ali MS. Saponins from *Zygophyllum probinquum*. *Phytochemistry* 1993; 33: 453–455
- 8 Attia AA. Triterpenoidal saponins from the aerial parts of *Zygophyllum coccineum* and *Zygophyllum fabago*. *Pharmazie* 1999; 54: 931–934
- 9 Ahmed VU, Uddin GS. A triterpenoid saponin from *Zygophyllum probinquum*. *Phytochemistry* 1992; 31: 1051–1054
- 10 Ahmad VU, Uddin GS, Bano S. Saponins from *Zygophyllum propinquum*. *J Nat Prod* 1990; 53: 1193–1197
- 11 Smati D, Offer ACM, Miyamoto T, Hammiche V, Dubois MAL. Ursane-type triterpene saponins from *Zygophyllum gestlini*. *Helv Chim Acta* 2007; 90: 712–719
- 12 Amin E, El-Hawary SS, Fathy MM, Mohammed R, Ali Z, Khan IA. Zygophylloside S, a new triterpenoid saponin from the aerial parts of *Zygophyllum coccineum* L. *Planta Med* 2010; 76: P51
- 13 Harput ÜS, Saracoğlu İ, Ogiyara Y. Methoxyflavonoids from *Pinaropappus roseus*. *Turk J Chem* 2004; 28: 761–766
- 14 Wedge DE, Kuhajek JM. A microbioassay for fungicide discovery. *SAAS Bull Biochem Biotechnol* 1998; 11: 1–7
- 15 Pridgeon JW, Becnel JJ, Clark GG, Linthicum KJ. A high throughput screening method to identify potential pesticides for mosquito control. *J Med Entomol* 2009; 46: 335–341
- 16 Gerberg EJ, Barnard DR, Ward RA. Manual for mosquito rearing and experimental techniques: Bulletin No 5. Mount Laurel: American Mosquito Control Association; 1994

received July 27, 2010

revised September 27, 2010

accepted September 30, 2010

Bibliography

DOI <http://dx.doi.org/10.1055/s-0030-1250463>

Published online October 26, 2010

Planta Med

© Georg Thieme Verlag KG Stuttgart · New York · ISSN 0032-0943

Correspondence

Ikhlas A. Khan, PhD

Research Institute of Pharmaceutical Sciences and Department of Pharmacognosy, School of Pharmacy National Center for Natural Products Research, The University of Mississippi University, MS 38677 USA
Phone: + 166 29 15 78 21
Fax: + 166 29 15 70 62
ikhlan@olemiss.edu